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The Agricultural Chemical Society of Japan.

President : Umetarō SUZUKI.

The Council of the Agr. Chem. Soc. of Japan has decided to publish English Abstract of those papers appearing in the Journal in a separate form in order to facilitate the circulation in foreign countries.

Bulletin of the Agr. Chem. Soc. of Japan is published for this purpose from May 1926 monthly. The numbering begins with Vol. 2, No. 5. The earlier parts are represented by the English abstracts published in the Journal annexed to the Japanese texts.

The articles to be appeared in the Bulletin must be concise, supplied with experimental methods and data and understandable, without specially referring to the Japanese texts. It ought, however, not exceed four printed pages as a rule. Any longer articles may be accepted according to the decision of the Council, with or without charge for exceeding pages.

Journal of the Agr. Chem. Soc. of Japan will be published in Japanese as formerly. Those desiring the detailed information of the articles appeared in the Bulletin may look for in the Journal of the same Number or the same Volume.

ON THE SOME NITROGENOUS CONSTITUENTS OF THE LEAVES OF KUZU (*The Japanese arrow-root plant, Pueraria hirsuta, Matsum.*). (Continued)

By

RINJIRO SASAKI

(Received Sept. 5th., 1927)

In the preliminary report,[1] the data were given of the protein of the japanese arrow-root plant. The author continued the experiment and attempted to determine the other constituents of the leaves. And two bases, two amino acids and one fatty acid were isolated from the filtrate, that was obtained from the precipitate of protein produced by neutralizing the alkaline alcohol extract of the leaves.

EXPERIMENTAL

Procedure and Method

The filtrate obtained from the precipitate produced by adding dilute hydrochloric acid to the alkaline alcohol extract[2] of the sample (the powdered leaves of the japanese arrow-root plant), was allowed to settle in the cold. The crystalline precipitate was produced. This was separated by centrifuging from mother liquid, and washed with the mixture of absolute alcohol and ether.

The mother liquid and washings were mixed and concentrated to about 1000 ccm. under the reduced pressure. After almost all of alcohol and ether were driven off, the remained solution was treated in the following manner.[3]

Pb-acetate (Ppt. I) | Filt. A

Filt. A: Neuberg's reagent (Ppt. II) | Filt. B

Ppt. II: P-tungstate (Ppt. III) | Filt. B

Filt. B: $HgCl_2$ (Ppt. IV) | Filt. C

After neutralizing, 20% solution of basic lead acetate was added to the above filtrate until no more precipitate was produced (Ppt. I).

Filtrate A :– The filtrate separated from the white precipitate was neutralized with acetic acid using lithmus as an indicator, and lead was removed by hydrogen sulfide as lead sulfide. The clear filtrate was freed from hydrogen sulfide on the boilng water bath and concentrated to 1000 ccm.

Then 20% Na_2CO_3 and 20% $HgCH_3COO$ (The Neuberg's reagent) were alternately added to this concentrated solütion until orange precpitate was produced. Same volume of 90% alcohol was added to the solution and the precipitate was separated by centrifuging. The precipitate was washed repeatedly with 50% alcohol.

The precitate (Ppt. II) thus obtained was suspended in water and mercury was removed by hydrogen sulfide.

The mother liquid of the precipitate II and washings were mixed and concentratrd to 1000 ccm. under the diminished pressure (Filt. B).

Precipitate II :– After the mercury free solution from the precipitate II was acidified to 5 per cent with sulfuric acid, 20% phosphotungstic acid was added to complete the precipitation. The precipitate (Ppt. III) thus obtained was washed with 5% phosphotungstic acid and separated by centrifuging (Filt. D).

Filtrate B :– Filtrate B was acidified with acetic acid and freed from mercury by hydrogen sulfide. The solution was acidified to congo red with hydrochloric acid. Then the solution was concentrated in vacuo, and sodium chloride and other inorganic salts were removed repeatedly by absolute alcohol. The filtrate was concentrated to about 300 ccm. Then hot alcoholic saturated solution of $HgCl_2$ was added and the precipitate produced was separated by centrifuging.

This process was repeated. The precipitate (Ppt. IV) thus obtained was treated with hydrogen sulfide and mercury was removed as mercury sulfide.

Separation of Purine Base

Baryta was added to the phosphotungstic acid precipitate (Ppt. III) in excess and the residue was digested three times with hot baryta to remove the phosphotungstic acid as barium salt.

The excess of baryta in the solution was removed as sulfate.

The solution thus obtained was concentrated to syrup. On cooling, some crystalline substance appeared. Then the syrup was dissolved in water to concentrated solution and faintly acidified with nitric acid. On adding $Ag\ NO_3$ a dark brown precipitate was produced, but the yield was very little.

After the precipitate was treated with ammonia and silver was removed by hydrochloric acid, the filtrate was concentrated.

The half of it was evaporated and the excess of hydrochloric acid was

driven off, and the picrate was thrown down from the solution by the addition of sodium picrate.

This preparation consisted of yellow fine needles grouped in bunches. The solubility of it in water and cold alcohol is very little, but easily soluble in hot water and alcohol.

It darkens at 281°C and melts at 290°C with decomposition evolving an yellow vapour.

From the other half of the solution, an orange crystalline chloraurate was produced by the addition of chloro-auric acid.

This darkens and melts at 218°C to a very viscous liquid.

Analysis :–

Substance 0.0850 gm.
0.0339 gm. Au = 39.84 % Au
Calculated for Adenin chloraurate ($C_5H_5N_5HCl \cdot AuCl_3 \cdot H_2O$)
39.98 % Au

The melting points of these salts and analyses indicate the salts of adenin.

Excess of $AgNO_3$ and baryta were alternately added to the above mother liquid, that was filtered from the precipitate produced by $AgNO_3$ in the acidified solution, and the precipitate was separated.

Then the above precipitate was decomposed by hydrochloric and sulfuric acid and examined by the usual method. When chloro-auric acid was added to the concentrated solution of free base, an orange leaflet crystal was formed.

This melts at 117–118°C distinctly. It was not able to determine further in detail, for the amounts obtained were too small.

The effort of separation of hexone bases, cholin or betain was in vain.

Separation of Amino Acids

Filtrate D was treated with baryta and freed from sulfuric and phosphotungstic acid, and then excess of baryta was removed quantitatively by sulfuric acid in the form of barium sulfate.

The clear filtrate thus obtained was concentrated to 40 ccm. under the diminished pressure. On being allowed to settle in the cold, a hexagonal crystal was formed in the solution.

This was purified and converted into copper salt by copper acetate. The copper salt crystallized in the form of rhombic plate.

Analysis :–

Substance 0.1345 gm.
0.0327 gm. CuO = 0.0261 gm. Cu
= 19.40 % Cu
Calculated for Asparagine-copper ($C_8H_{14}N_4O_6Cu$)
19.52 % Cu

The result of analysis indicates asparagine.

The filtrate obtained from asparagine crystal was concentrated and saturated with hydrochloric acid. On cooling, much amount of crystal was separated.

This melts at 205°C. The copper salt of it crystallized in the bushy form of needles.

Analysis :–

Substance 0.1524 gm.

0.0571 gm. of CuO = 0.0456 gm. Cu

= 29.95 % Cu

Calculated for Copper-glutamate ($C_5H_7NO_4 \cdot Cu \cdot \frac{1}{2}H_2O$)

29.21 % Cu

The melting point and analyses indicate glutamic acid.

Separation of Organic Acid

Filtrate C was freed from alcohol and mercury by the usual method. By the extraction of the filtrate with ether, an oily fatty acid was obtained.

The half of it was converted into copper salt with copper carbonate. The copper salt crystallized in hexagonal plates.

The other half was converted into silver salt. On recrystallization, the silver salt crystallized in the bushy form of needles mixed with long plate.

Analysis :–

Substance 0.1123 gm.

0.0374 gm. CuO = 0.0299 gm. Cu

= 26.62 % Cu

Calculated for Copper butyrate ($C_8H_{14}O_4Cu$)

26.75 % Cu

The cystal forms of its salts and analyses indicate butyric acid.

It is not certain that whether butyric acid is a natural constituent of the leaves or a fermentation product produced during drying.

Summary

The nitrogenous constituents of the japanese arrow-root plant "Kuzu" have been studied with the following results.

1) A protein was obtained from the alkaline alcohol extract of the sample by neutralizing with dilute hydrochloric acid. It contains 14.2% of nitrogen. Its distribution of nitrogen is as follows : Arginine N 8.80%, Cystine N 0.14%, Histidine N 4.63%, Lysine N 2.00% and N of Mono-amino acid 48.14% in total nitrogen.

2) The filtrate obtained from the precipitate produced by adding dilute

hydrochloric acid to the alkaline alcohol extract of the sample was further examined, and two bases, two amino acids and one fatty acid were obtained.

Those are adenin, a base yielding a chloraurate melting at 117–118°C, asparagin, glutamic acid and butyric acid.

3) An unknown crystalline nitrogenous substance, melting at 185–187°C, was obtained. But the amount was too small to permit of identification.

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BIOLOGICAL EFFECT OF ULTRAVIOLET RAY UPON THE SILKWORM (BOMBIX MORI).

By

Shuiku Sasaki and Osho Kei.

(Received Sept. 29th, 1927.)

(From the Biochemical and Sericultural Laboratories, Department of Agriculture, Kyushu Imperial University.)

The present paper deals with some of the experimental results of the biological effects of ultraviolet ray upon the silkworm.

1. Permeability of cuticula of the larva.

Before experimenting for the biological effects, we examined whether the ultraviolet ray could penetrate cuticula of the silkworm or not, and the following results were obtained, using a Hilger quartz spectrograph which was situated at a distance of 50cm. from the burner of a quartz mercury lamp (6 amps., 110 volts). Cuticula was placed before the slit of the spectrograph.

a). The permeability of cuticula of the silkworm was almost equal to that of the chicken and albino rat.

b). They permitted the passage of the ray of wave length longer than 2950 Å for 2 minutes.

2. Biological effects on Silkworms.

Silkworms were placed in a water-jacketed box and the inner temperature of it was kept at 26 ± 1.5C., and the surrounding air of the worms was

gently replaced by fresh one by means of a pump throughout the time of irradiation. The ultraviolet ray was radiated from the quartz mercury vapour lamp before mentioned and filtered by a glass filter which permitted the passage of an ultraviolet ray of a wave length of between 3100 Å to 3800 Å. The distance between the silkworms and the burner was 30cm.

a). Three groups of the larvae were irradiated daily for 15, 30 and 60 minutes respectively during a period of breeding. Although the effects of the ultraviolet ray were not remarkable, the health of the worms was promoted, the rate of death decreased generally in proportion to the length of time of irradiation, and the weights of the larva, the cocoon and the silk obtained were increased by the short time irradiation but decreased by the long time.

b). The larvae of the silkworm named "opeck" which have transparent skins produced some violet spots on their skins in a few days after irradiation.

c). The pupae plucked out from cocoon layers were irradiated for 2 days, but they did not suffered any abiotic effects.

d). One group of the eggs of silkworms was irradiated for 9 days for 20 minutes daily, and another for 15 minutes at the beginnig of the experiment, the time being gradually increased up to 2 hours. No injurious effects were observed in either groups when they were incubated, but the latter showed a slightly better result in the percentage of the death rate of the larvae in breeding.

STUDIES ON BIOS. PART III.

By

BUNSUKE SUZUKI and YASUJI HAMAMURA.

(Received Oct. 6th., 1927.)

Crude bios[1] was treated with a strong (30%) caustic soda solution, at boiling temperature, for 3 hours and to the reaction mixture a large amount of pure alcohol was added. On standing over night, a resinous matter separated out this being easily removed by decantation. The clear solution thus obtained was submitted to distillation under reduced pressure until its volume about one half of the orginal. On standing over one night or more, yellowish crystalline plates separated out, proving to be sodium salt of an organic acid. The free acid, formed by adding dilute sulfuric acid to the aqueous solution

of the salt, was recrystallized from either hot water or benzene. This free acid contains neither S nor N and has the experimental formula of $C_{10}H_{10}O_5$ (m. w. found 216), to which we have given the name of γ–acid, for the time being, and it contains one carboxyl and hydroxyl and two methoxy groups. The acid itself is quite unstable but its methylated products become durable for oxidation by alkaline permanganate solution at room temperature, forming trimethyl gallic acid (I). In this respect it resembles the iridic acid $(C_{10}H_{12}O_5)$[2] (II) of G. de Laire and Tiemann. It might be suspected, therefore, that the organic acid is an isomeride of iridic acid.

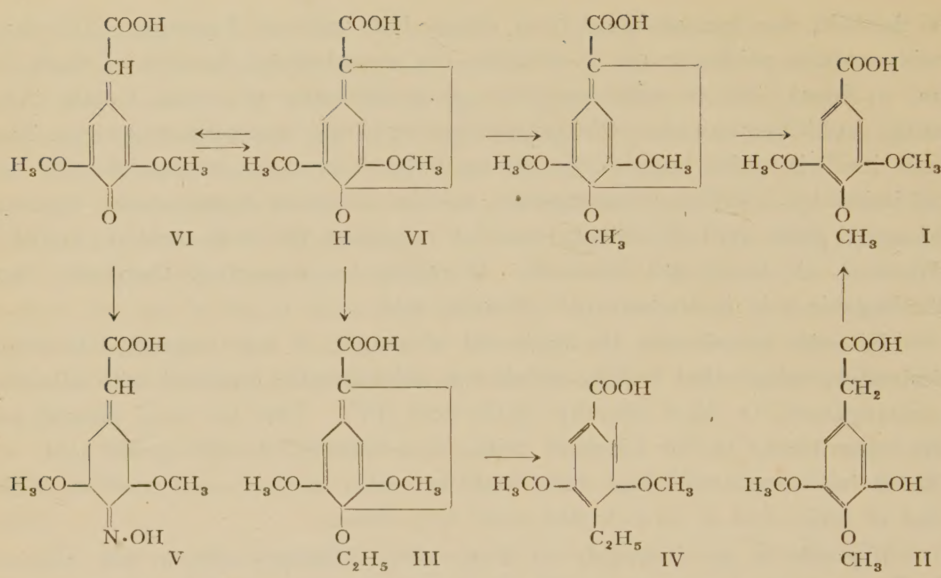
In order to orientate the hydroxyl of γ–acid, it was converted to ethyl derivative, using ethyl iodide, which was subsequently oxidized with alkaline permanganate, to ethyl dimethyl gallic acid (IV). This has been proved, on the other hand, to be identical with 3, 5–dimethyl–4–ethyl gallic acid, so that it has been established that hydroxyl of γ–acid is on 4 position while that of iridic acid is on 5 to the main side chain.

If γ–acid is an isomeride of iridic acid, differing only in the relative positions of the hydroxyl and methoxy groups, the methyl derivatives of both substances must be identical. But this is not the case and, moreover, the following facts. i. e. that the results of elementary analysis of γ–acid does not conform to the formula of $C_{10}H_{12}O_5$; that methyl γ–acid does not form a stable dibromo compound while iridic acid does; that on dry distillation methyl iridic acid converts to 3, 4, 5–trimethoxy–1–methyl benzene, which is not the case with γ–acid and that the methyl ester of methyl iridic acid is an oil while that of γ–acid is a crystalline substance, show that γ–acid differs also from iridic acid in regard to the form of other elementary groups.

By the action of hydroxylamine on γ–acid in acidic medium an oxime (V) is formed; the reaction, however, does not take place in an alkaline solution. It was assumed, therefore, that there are two tautomeric isomerides, keto–and enol–form, in γ–acid, in alkaline solution converting to enol–form.

Taking the following facts into consideration, i. e. that γ–acid is a coloring matter: that one hydroxyl, which lies on p–position to the main side chain, easily changes to ketone group and that, to conform with the results of analysis, one of two carbon atoms of the main side chain cannot take more than one atom of hydrogen, we assigned quinoid ring to γ–acid. (VI).

If γ–acid has the constitution of VI, it should be expected to obtain glyoxylic acid and dimethyl gallic acid or their derivatives, by acid hydrolysis. In fact formaldehyde was identified as one of the products, while an oily matter, which is considered to be a condensation product of quinoid substance or dimethyl pyrogallol, was found in the residue. Formaldehyde was produced, presumably, by the decomposition of glyoxylic acid.



Experimental

γ-acid. To 20g. of crude bios 100c.c. of a 30% caustic soda solution was added and kept at boiling temperature for 3 hours. The reaction mixture was filled up to 1 lt. with pure alcohol. On standing over night a resinous matter separated out which was removed by decantation. The clear solution thus obtained was submitted to distillation under reduced pressure until its volume became about one half of the original when yellowish brown crystalline plates have separated out. The free acid obtained by treating the salt with sulfuric acid was recrystallized either from hot water or benzene. M. p.: 187–189° (decomposing). m. w. found: 235 (by m. p. depression of camphor solution). Subst.: 0.0512g., CO_2: 0.1074g., H_2O: 0.0226g., C: 57.20%, H: 4.90% (C: 57.13%, H: 4.76% as $C_{10}H_{10}O_5$).

Benzoyl γ-acid. γ-acid was benzoylated by Schotten-Baumann's method using benzoyl chloride. M. p.: 216°C, subst.: 0.0544g, CO_2: 0.1300g., H_2O: 0.0232g., C: 65.17%, H: 4.73% [C: 64.96%, H: 4.45% as $C_{10}H_9O_5$ (C_6H_5CO)].

Methyl γ-acid. When γ-acid was methylated with di-methyl sulfate, 2 forms were produced. The one, which is neutral to lithmus, separated out from a neutral medium. M. p.: 100°C, subst.: 0.0498g., CO_2: 0.1096g., H_2O: 0.0264g., C: 60.02%, H: 5.89% [C: 60.50%, H: 5.88% as $C_9H_8O_2$ (OCH_3) $(COOCH_3)$].

The other was an acid which separated out from an acidic medium. M. p.: 130°C, subst.: 0.0505g., CO_2: 0.1104g., H_2O: 0.0234g., C: 59.62%,

H: 5.14% [C: 58.92%, H: 5.35% as $C_{10}H_9O_4$ (OCH_3)].

Ethyl ester of γ–acid. The ester was prepared by dissolving γ–acid in pure alcohol and passing dry HCl. M. p.: 86°C, subst.: 0.0488g., CO_2: 0.1094g., H_2O: 0.0268g., C: 61.14%, H. 6.10%. [C: 60.50%, H: 5.88% as $C_9H_9O_3$ $(COOC_2H_5)$].

Trimethyl gallic acid. 1g. of methyl γ–acid was dissolved in a 0.5% caustic soda solution and, under a thorough cooling and constant stirring, $KMnO_4$ solution (5%) was added drop by drop. Mangan oxide formed was filtered off, the filtrate was concentrated by distillation under reduced pressure and the solution was acidified when the substance crystallized out. M. p.: 169°C, subst.: 0.0469g., CO_2: 0.0976g., H_2O: 0.0242g., C: 56.75%, H: 5.73%, [C: 56.58%, H: 5.65% as $(CH_3O)_3$ C_6H_2 COOH].

Trimethyl gallic acid prepared synthetically from gallic acid had the same m. p. and the mixture of the two also melted at the same temperature.

Dry distillation of γ–acid. An oily substance was obtained by dry distillation of methyl γ–acid under reduced pressure. It decomposes at about 330°C without boiling, differing from 3, 4, 5, trimethoxy–1–methyl benzene obtainable by the simillar treatment of iridic acid. By oxidizing the substance with alkaline $KMn0_4$ trimethyl gallic acid was obtained.

Ethyl γ–acid. 2g. of γ–acid was dissolved in 30c.c. of 5% caustic soda, an excess of ethyl iodide was added to the solution and the reaction mixture was heated for 5 hours. The solution was cooled and was made acidic by adding sulfuric acid when colourless crystals separated out. Subst.: 0.0513g., CO_2: 0.1133g., H_2O: 0.0290g., C: 60.23%, H: 6.27% [C: 60.50%, H: 5.88% as $C_{10}H_9O_4$ (OC_2H_5)].

3, 5–*dimethyl*–4–*ethyl gallic acid.* Ethyl γ–acid was oxidized as methyl γ–acid. M. p.: 123°C, subst.: 0.0478g., CO_2: 0.1014g., H_2O: 0.0286g., C: 57.85%, H: 6.64% [C: 58.40%, H: 6.19% as $(CH_3O)_2$ (C_2H_5O) C_6H_2 COOH)].

Syringaic acid (3, 5–dimethyl gallic acid) was synthesized by the method of Graebe and Martz and was ethylated with ethyl iodide, the effect being that the product (3, 5–dimethyl ethyl gallic acid) was proved to be the same substance as that obtained from ethyl–γ–acid showing the same composition, and the same m. p. No alteration of the m. p. by mixing the two, was observed.

Dihydro γ–acid. γ–acid was reduced in an alcoholic solution with Willstätter's method, and dihydro–γ–acid was obtained. M. p.: 102°C., subst.: 0.0511, CO_2: 0.1059g., H_2O: 0.0256., C: 56.52%, H: 5.56% [C: 56.58%, H: 5.70% as $C_{10}H_{12}O_5$].

Dihydro–methyl–γ–acid. Dihydro–γ–acid was oxidized with alkaline per-

manganate. M. p.: 105°C, subst.: 0.0493g., CO_2: 0.1058g., H_2O : 0.0271g., C: 58.52%, H: 6.10% [C: 58.40% as $C_{10}H_{11}O_5 \cdot CH_3$].

This substance is quite stable aganist any oxiding agent alkaline permanganate showing no trace of oxidation.

Dihydro dibromo methyl γ-acid. Dihydro methyl γ-acid was dissolved in glacial acetic acid and was brominated. M. p.: 122°C, subst.: 0.1005g., AgBr: 0.0984g., Br: 41.67% [Br: 41.63% as $C_{11}H_{12}O_5Br_2$].

Oxime. 3g. of γ-acid, 2.5g. of hydroxylamine hydrochloride and 3.5g. of sodium acetate were dissolved in 80c.c. of 60% alcohol and the solution was heated for 3 hours on a water bath. Colorless plates separated out. The substance had no melting point, decomposing at 199–201°C. N: 5.95% [6.22% as $C_{10}H_{11}O_5N$].

A part of the expenses of the study was provided by this Academy for which we express our sincere thanks.

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ON THE REVERSIBILITY OF ENZYME ACTION. PART I.

By

Bunsuke Suzuki and Tsutomu Maruyama.

(Received Oct. 6th., 1927.)

Armstrong[1] found that, by the action of yeast maltase on glucose, isomaltose mainly was formed and has taken the view that an enzyme synthesizes exactly those bodies which it does not hydrolyse. To accept this view, it is a matter of primary importance to ascertain whether the maltase preparation used by Armstrong really did not contain any β-glucosidase. On reading Armstrong's papers, one cannot get any information about this matter either positively or negatively.

The observation of the presence of β-glucosidase in yeast cells (brewer's yeast), made by Henry and Auld,[2] is well founded and Pringsheim[3] noticed that maltose and a β-glucoside are formed by the action of yeast enzymes. It is very likely that these experiments present an argument against Armstrong's view.

If it were possible to have in hand a maltase preparation free from β–glucosidase the matter would be settled, once for all. Unhappily, however, maltase is weaker than β–glucosidase against acids which are to be produced during the autolysis of yeast cells, so that it is quite difficult to obtain such an enzyme preparation. It is an easy task, on the contrary, to prepare, by taking advantage of this property, β–glucosidase solution free from maltase.

Using the latter preparation we were able to prove that isomaltose is formed by its action upon a concentrated glucose solution. Isomaltose was identified as its osazone, comparing it with that of isomaltose synthesized, following Fischer's method. When the synthesis was completed, that is when the change of rotatory and reducing power was at a standstill, the solution was diluted and the recovery to their original state was observed.

Since maltase was not contained in the enzyme preparation which we used, maltase, without doubt, dose not play a role in the reaction. Neverthless isomaltose was formed. It is to be expected naturally that our enzyme preparation may contain other carbohydrases than β–glucosidase, for instance, invertase. It is contrary, however, to the general conception of the nature of enzyme action to suspect them to be responsible for the synthesis of isomaltose. It has been thought, therefore, that isomaltose was formed, in our experiments, by the action of β–glucosidase upon glucose.

Experimental

A dry preparation of yeast was prepared by treating beer yeast, supplied to us from Dai Nihon Brewering Co., with alcohol and ether successively after it was well centrifuged to drive water. To 10g. of the dry preparation, 100c.c. of water and 5c.c. of toluene were added and the mixture was well macerated in a mortar. Standing over night at room temperature (18–20°C), the mixture was filtered with Chamberland's filter and the filtrate was used for experiments after being neutralized with a 1% ammonia solution.

1. *Action on maltose.* 1.26g. of maltose were dissolved in 70c.c. of water and to the solution 7c.c. of enzyme preparation and 10c.c. of buffer solution were added. Secondary sodium phosphate and primary potassium phosphate were used as buffers. For observation 1c.c. of the mixed solution was used. pH : 6.8, temp. : 37°C. No change of rotatory and reducing power was observed during the course of 168 hours.

2. *Action on α–methyl glucoside.* All conditions, except the concentration of the substrate, were the same as those of case 1. Concentration of glucoside was 1.5g. in 35c.c. of reaction mixture. No change of rotatory and reducing power was observed.

3. *Action on β–methyl glucoside.* The reaction mixture consisted of the

following solution: 15c.c. of the glucoside solution (0.5g.), 5c.c. of enzyme preparation, 1.18c.c. of 0.2N acetic acid and 2.82c.c. of 0.2N sodium acetate solution. pH: 5.0, temp.: 37°C. After 58 hours, 1c.c. of the solution precipitated cuprous oxide to the amount of 61.37mg. as copper.

4. *Action on isomaltose.* An isomaltose solution was prepared after Fischer's method. pH: 5.1, temp.: 37°C. 1c.c. of the reacting solution precipitated 51.81mg. of Cu in the beginning and after 90 hours the precipitates increased to 61.37mg. of Cu.

As the isomaltose solution might contain other di– or polysaccharides than isomaltose, isomaltosazone was formed and the action of the enzyme preparation on the osazone was observed.[5] 0.01g. of the osazone, which naturally did not show any reducing power, precipitated 4.02mg. of Cu 3 days after the enzyme solution had been added. Control experiments which, however, showed no change were duly carried out, side by side.

5. *Action on glucose.* Concentration of glucose: 52.8%, pH: 6.4, temp.: 37°C. The decrease of reducing power was observed and when the same solution was diluted as much as five times the original reducing power was recovered.

One part of the synthesized solution was taken out, diluted with water to make the concentration of sugar 10%, pasteurized as usual, and impregnated with S. marxianus. When fermentation ceased, an osazone was formed by the ordinary method. M. p.: 155°C. C: 55.75% (Calc.: 55.35), N: 10.57% (Calc.: 10.77). A mixed sample of the osazone and isomaltosazone, obtained after Fischer's method, melted also at 155°C.

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VERSUCHE ÜBER DIE GÄRUNGSPRODUKTE MITTELS SCHIMMELPILZE.

III. CYTOSPORELLA DAMNOSA.

von

YUSUKE SUMIKI.

(aus dem Laboratorium der Agrikulturchemie an der Staats-Universität in Tokyo.)

(Angenommen am 31, Okt., 1927)

Cytosporella damnosa wird in der folgenden Nährlösungen (13 L.) bei 30°C während 28 bis 40 Tagen gezüchtet.

Glykose	10.0 g
Pepton oder $(NH_4)_2SO_4$	0.100 〃
KH_2PO_4	0.015 〃
K_2HPO_4	0.015 〃
$MgSO_4$	0.010 〃
$CaCl_2$	0.010 〃
NaCl, $FeCl_3$	Spur
H_2O	100.0 〃

Isolierung und Erkennung von Gärprodukte.

Durch Destillation mit Wasserdampf, die flüchtige Substanzen in vergörenen Nährflüssigkeiten werden von den nichtflüchtige Substanzen geschieden. [Rückstand (A), Destillat (B).] Man erhitzt das Destillat (B) am Rückflusskühler mit dem Zusatz von Bariumkarbonat, filtriert es, und das Filtrat wird zur Trennung die Alkohole und die Aldehyde von den Bariumsalzen der flüchtige Säuren destilliert. [Rückstand (C), Destillat (D)]

(1) Aethylalkohol.

Eine Fraktion, sd. Pt. 78°C, wird durch wiederholte fraktionierte Destillation des Destillats (D) gewonnen. Aethylester der Phenylcarbaminsäure, schmz. Pt. 56°C, entsteht aus dieser Fraktionen durch Behandeln mit Phenylisocyanat.

0.0810g Subst. gaben 5.75cm³ N_2 (755.5mm., 9.5°C).
$C_9H_{11}NO_2$ Ber. N 8.49%.
Gef. N 8.45%.

(2) Fuselöl.

Der qualitative Nachweis erfolgt durch die Farbenreaktionen mit dem Destillate (B).

a) Furfurol-reaktion nach Jorissen (Ber., **13**, 2439, 1880).
b) Salicylaldehyd-reaktion nach Komorowski (Chem. Ztg., **27**, 808, 1903).
c) Vanillin-schwefelsäure-reaktion nach Takahashi (Ztsch. f. Nähr. u.

Genuss., **27**, 820, 1914).

(**3**) Acetaldehyd.

Durch wiederholte fraktionierte Destillation des Destillats (D), wird eine Fraktion gewonnen, die Rotfärbung mit fuchsin-schweferiger Säure nimmt. Aus dieser Fraktionen, schied sich p-Nitrophenylhydrazon des Acetaldehyds, schmz. Pt. 128°C, ab durch Behandeln mit p-Nitrophenylhydrazin.

0.0310g Subst. gaben 6.0cm³ N_2 (763.5mm., 9°C).
$C_8H_9N_3O_2$ Ber. N 23.46%.
Gef. N 23.27%.

(**4**) Bernsteinsäure.

Der Rückstand (A) wird auf dem Wasserbad eingeengt, angesäuert mit Phosphorsäure und dann extraktiert mit Aether. Nach Entfernung des Aethers, Bernsteinsäure, schmz. Pt. 183°C, wird gewonnen. Und es im Wasser löst, und wird dem mit Natronlauge neutralisierte Lösung von Bernsteinsäure mit Silbernitratlösung zugefugt, so entsteht der starke Niederschlag von Silbersuccinat.

0.9828g Subst. gaben 0.8388g ClAg.
$C_4H_4O_4Ag_2$ Ber. Ag 65.03%.
Gef Ag 64.13%.

(**5**) Essigsäure.

Man säuert den Rückstand (C), welcher auf dem Wasserbad fast zur Trockne verdampft wurde, mit Schwefelsäure und abdestilliert. Aus diesem Destillate, durch Behandeln mit Silbernitrat, bild sich Silberacetat.

0.0958g Subst. gaben 0.0804g ClAg.
$C_2H_3O_2Ag$ Ber. Ag 64.64%.
Gef. Ag 63.15%.

Quantitative Bestimmung der Gärprodukte.

Die Menge der Säuren in vergörenen Flüssigkeiten wird durch Titrierung mit 1/10 N Natronlauge bestimmt.

Der Alkoholgehalt in vergönnen Flüssigkeiten wird aus dem spez. Gewicht mittels des Piknometers bestimmt.

Der Glykosegehalt wird nach der Methode von Bertrand bestimmt.

Tage nach Einimpfung	Alkohol gehalt (vol%)	Säuregehalt in 100cm³ als Bernsteinsäure (cg)	Glykosegehalt in 100cm³ (g)
4	0.13	7.01	9.02
6	0.33	9.34	8.88
8	0.70	10.51	8.37
10	0.87	14.02	8.05
12	1.34	14.85	7.84
14	1.88	16.35	6.23
16	2.09	15.19	5.91
18	2.37	20.73	5.62

20	3.49	22.78	3.98
22	3.56	22.78	3.72
24	3.64	22.78	3.05
26	3.28	22.78	2.02
28	3.21	21.09	2.02
30	2.72	21.03	1.94

Tage nach Einimpfung	Alkohol (g) aus 100g Stärke	Alkohol (g) aus 100g Reise
21	10.67	—
31	8.50	—
38	—	7.03
41	7.83	4.17
51	10.67	—

(August, 30, 1927)

ON THE PHYSIOLOGY OF RHIZOPUS SPECIES. III.

PART X. VERIFICATION OF ZYMASE AND CARBOXYLASE BY LOHNSTEIN'S FERMENTING TUBE.

By

TEIZO TAKAHASHI and TOSHINOBU ASAI.

(Received Nov. 4th., 1927.)

It has already been established by the previous works by one of us (T. T.) and his co-workers.[1] that the occurrence of zymase in Rhizopus species was verified just by the formation of alcohol, lacking thereby the evidence of production of CO_2 by both cases of enzymes viz. zymase and carboxylase, Its main cause being likely due to the deficiency of the construction of Einhorn's fermenting tube, we changed the scheme in this experiment to use Lohnstein's one. By this device the verification of both enzymes stated, was put forwarded strictly by the way of the formation of CO_2 from glucose and pyruvic acid.

Experimental

Far the culture of Rhizopus species "Koji"-extract was taken as culture medium to which 0.4% of peptone was added for the purpose of to promote the formation of zymase. Through whole period of cultivation, care was always taken to prevent the formation of spores and to get so-called immersed culture by shaking the flask very often, generally twice every day.

Acetone–fungus viz. the preparate[2] of fungus treated by acetone was taken in such a weight of 3 grams for each determination and to this two kinds of solutions were added separately. One was the solution of glucose and the other one containing some salts beside glucose as buffer, as shown below :—

I).	Glucose	10 %.	pH = 6.0
	n/15 KH_2PO_4.	18 c.c.	
	n/15 Na_2HPO_4	2 c.c.	
	Water	80 c.c.	
II).	Glucose	10 g.	
	Water	100 c.c.	

The mixtures of acetone–fungus and the solution mentioned above were filled in Lohnstein's fermenting tube with association of 0.1c.c. of toluol, making six series of procedures in all, and held at 25–30°C. The results are tabulated below :—

	Acetone-fungus.	Toluol.	Evolution of CO_2, indicated by elevation of Hg, column, after.			
I. Solution I.			16 hours.	40 hours.	65 hours.	80 hours.
〃 a.	3 g.	0.10 c.c.	0.2 m.m.	0.4 m.m.	0.6 m.m.	0.6 m.m.
〃 b.	3 〃	〃	0.4 〃	0.5 〃	1.5 〃	1.5 〃
II. Solution II.	3 〃	〃	0.2 〃	0.3 〃	0.6 〃	0.6 〃
III. Solution I.	3 〃	non	0.4 〃	0.5 〃	1.7 〃	1.7 〃
IV. Solution I. (boiled).	3 〃	0.1 c.c.	—	—	—	—
V. Solution II.	non	〃	—	—	—	—
VI. Sterile water.	3 g	〃	—	—	—	—

Remarks :– At the end of observation to ascertain the nature of gas, it was discharged from the side tube (specially attached by our case) of the lower part of apparatus, and introduced into dilute aqueous solution of NaOH associated with phenolphthaleine.

For the affirmation of carboxylase, two solutions of different pH stated below were prepared :—

Solution I.

Pyruvic acid	0.7 g.	pH = 4.0
K_2HPO_4	1.2 g.	
Distilled water (sterile)	100 c c.	

Solution II.

Pyruvic acid	0.7 g.	pH = 3.0
K_2HPO_4	0.53g.	
Distilled water (sterile)	100 c.c.	

The results are shown below :—

1) and 2). Bulletin of Agric. Chem. Soc. Japan. Vol. 3 Nos. 31–35 p. 87, Teizo Takahashi, Kinichiro Sakaguchi and Toshinobu Asai.

	Acetone-fungus.	Toluol.	Evolution of CO_2, indicated by elevation of Hg-Column, after			
			16 hours.	40 hours.	65 hours.	80 hours.
I. Solution I.	3 g.	0.1 c.c.	+	0.2 m.m.	0.4 m.m.	0.4 m.m.
II. Solution II.	〃	0.1 〃	+	0.2 〃	0.2 〃	0.3 〃
III. Solution I. (boiled).	〃	0.1 〃	—	—	—	—
IV. Solution II.	non	0.1 〃	—	—	—	—
V. Sterile water	3 g.	0.1 〃	—	—	—	—

Preparation of pressed juice.

The method of preparation of pressed juice from fungus is quite same as stated in previous paper[3] with exception of the exposure of chopped pieces of fungus to liguid carbondioxide prior to the grinding.

By this way from 300g. of fungus growth there comes forth 80c.c. of pressed juice. Accordingly, 10c.c. of the juice applied to each determination would correspond to about 4 grams of fungus mass.

To this juice either glucose or pyruvic acid was added for the verification of zymase or carboxylase with association of buffer. They are mentioned below :—

The solutions for zymase.

I. Solution.

Glucose 20 %
n/15 KH_2PO_4 1.8 c.c. } (pH = 6.2) } Fill up to 10c.c. with pressed juice.
n/15 Na_2HPO_4 0.2 c.c.

II. Solution.

Glucose 20 %
Pressed juice 10 c.c. }

The solutions for carboxylase are ;

III. Solution.

Pressed juice 10 c.c.
Pyruvic acid 0.7 % } pH = 4.0
K_2HPO_4 1.2 %

IV. Solution.

Pressed juice 10 c.c.
Pyruvic acid 0.7 % } pH = 3.0
K_2HPO_4 0.53%

The results are tabulated below :—

Zymase.	Toluol.	Evolution of CO_2, indicated by elevation of Hg-Column, after			
		16hours.	40hours.	65hours.	70hours.
I. Solution a.	0.1 c.c.	1.0 m.m.	1.5 m.m.	1.5 m.m.	1.6 m.m.
b.	〃	0.9 〃	1.1 〃	1.2 〃	1.2 〃

3) Bulletin of Agric. Chem. Soc. Japan, Vol. 3, Nos. 34-35, p. 87, Teizo Takahashi, Kinichiro Sakaguchi and Toshinobu Asai.

II. Solution	0.1 c c.	0.7 m.m.	0.7 m m.	0.8 m.m.	0.8 m.m.
I. Solution (boiled)	〃	—	—	—	—
Glucose solution 20%	〃	—	—	—	—
Just pressed juice	〃	+	0.2 m.m.	0.2 m.m.	0.2 m.m.

Carboxylase,

	Toluol.	16 hours.	40 hours.	65 hours.	70 hours.
II. Solution.	0.1 c c.	0.2 m.m.	0.3 m.m.	0.5 m.m.	0.5 m.m.
IV. Solution.	0.1 〃	0.2 〃	0.2 〃	0.5 〃	0.5 〃
III. Solution (boiled).	0.1 〃	—	—	—	—
Pyruvic acid solution (0.7%) with buffer and pH = 3.0	0.1 〃	—	—	—	—
Just pressed juice.	0.1 〃	+	0.2 m.m.	0.2 m.m.	0.2 m m.

Remarks :– To affirm the nature of the gas evolved, the same care as stated above was not neglected.

ON THE ORIGIN OF ALDEHYDES IN FERMENTATION PRODUCTS. III.

By

Masakazu Yamada.

(Received Nov. 10th., 1927)

Among the aldehydes, excepting the acetaldehyde, obserbed in fermentation products, the butyr-and valer-aldehyde were confirmed to have their sole origin in the oxidation of the corresponding alcohol by microbes.[1] In tht case of saké, the higher alcohols or fusel oil are ordinarily produced in a so small quantity that no appreceable oxidation products or aldehydes have yet been found in it, though once 360 liter of saké have been treated. As regards the furfurol it has been a question for many years since Foerster[2] whether the aldehyde is formed by heating or by real fermentation.

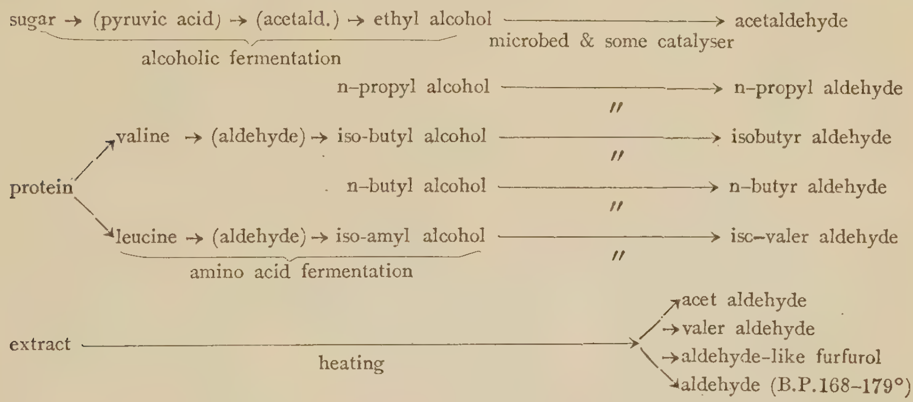
The authôr found that a considerable quantity of aldehydes was produced from the extract—distilling residue—of such fermentation products as the saké, the shōyu (soya sauce), the beer etc. by distillation in steam and that the aldehydes consisted of acet-, valer-, furfurol-like aldehyde etc. The larger the sp. gr. of the extract is, the better yield of aldehydes is obtainable. As the ethereal extract of the saké or shōyu at the end of fermentation

(1) M. Yamada : Bull. Agr. Ch. Soc. Jap., **3**, 80–3, 1927.

(2) Foerster : Ber., 15, 322, 1882.

shows no Jorissen's furfurol reaction with the aniline-acetic acid, it is not difficult to suppose that the so-called furfurol in fermentation products is produced along with other aldehydes from the extract by pasteurization or distillation. The reaction is traceable in the pasteurized saké and also in the unpasteurised, when the latter is heated at 40°C for half an hour. In the shōyu on the market the reaction is distinct, because its pasteurizing temperature lies generally above 60°C.

This fact explains the main origin of the valer-and furfurol-like aldehyde in the shōyu, raw spirit[3] and also several distilled alcoholic drinks. The origins of aldehydes in fermentation products without the use of fixatives, may, therefore, be shown in the following diagram.



The so-called furfurol does not comply with the furfurol as Kodama's view.

Experimental

I. Aldehydes, formed in the process of heating of the extract of the saké.

i. Preparation :-

The distilling residue of the saké is evaporated on the water bath into the syrup. The latter is again distilled in steam as long as the distillate shows a deep red colour with Schiff's reagent. The aldehydes are distilled in a quantity of 0.01–0.03% in the distillate. The distillate is again concentrated by repeated distillation, until a greenish yellow oil with a distinctive smell appears in the distillate. The whole is shaken with ether and the aldehydes are completely separated. After the dehydration and evaporation of the ether, the residue is fractionated according to the B. P., 184L. of saké were thus treated in two unequal parts.

ii. Properties :—

(3) Ordonneau : Z. Spirit. Ind., 11, 183, 1888.

Fract.	B. P.	Yield g. I	Yield g. II	Colour.	Rimini's test	Furfurol react.	P-nitro-ph. M.P.	P-nitro-ph. color	Aldomedon. M.P.
1	–40	0.45	1.7	colorless	++	–	122	orange	–
2	40–45	2.2	6.8	faint yellow.	+	–	–	〃	–
3	45–55	2.0	3.0	〃	±	–	101	〃	–
4	55–66	1.5	5.0	〃	++	–	101	〃	136
5	66–80	5.5	6.1	greenish yellow.	–	–	107	〃	141–5
6	80–94	1.8	1.3	〃	–	±	–		–
7	94–120	0.5	0.3	〃	–	+	–		–
8	120–160	0.7	–	yellowish brown	–	++	–		–
9	160	0.3	0.9	〃	–	++	130	red	resin
10	160–120	0.3	0.1	〃	–	++	–		–
11	residue	1.8	2.3	brown	–	++	–		–

iii. Analysis of p-nitrophenyl hydrazones.

Fract.	Sub. (g)	N. c.c.	T.	P.	N. found	N. calculated for	
3	0.1098	19.3	24°	758.5	19.73	20.30	$C_{10}H_{13}N_3O_2$
4	0.1227	20.8	23	761.4	19.19	19.00	$C_{11}H_{15}N_3O_2$ (valer-)
5	0.1104	18.5	18	753.8	19.26	〃	〃
9	0.1004	16.2	19	753.6	18.45	18.20	$C_{11}H_9N_3O_3$ (Furol)

Schiff's reaction, reduction of amm. silver-nitrate solution are positive in all fractions, Na-nitro prusside reaction by Rothera is negative. All fractions do not contain N and S. Only the fractions which give furfurol reaction decolorize bromine water. After standing for 2 months, a part of the fraction 5 converted into an acid, which boiled at 160–180° and had a similar odour to butyric acid, and did not mix with water easily. The silver salt crystallizes in colorless brilliant scales.

Sub. 0.1068 g. Ag 0.0552 g. Ag % found 51.68 Calc. 51.63 ($C_5H_9O_2Ag$; Valer.)

II. Aldehydes in the shōyu.

i. Aldehydes, produced in the real fermentation.

Schiffs reaction is positive in the ethereal extract of shōyu, but negative in the chloroform extract. This shows that only the aldehydes of lower B. P. exist. Air was passed in the non-pasteurized shōyu at 30°C for 8 hours and the volatile substances were all caught in two ice-cold sulfite solutions. The derivative of the aldehyde prepared from the solution are following.

Aldomedon. M. P.	p-nitro phenylhydrazone M. P.	p-nitro phenylhydrazone N found	N calculated
136°	121°	23.24	23.46 ($C_8H_9N_3O_2$)

Thus, only the acetaldehyde was detected in a some what impure state.

ii. Aldehydes, produced by heating.

After the removal of volatile constituents originally existed by distillation

in steam, the residue was treated in the same way as the case of the saké. Thus, the ether extract of the distillate, which came from 30L. of the shōyu and amounted to about 292L, was fractionated.

Main fract.	B. P.	yield g.	Rimini's test.	Furfurol react.	p-nitro ph. M. P.	p-nitro ph. color.	p-nitro ph. N %	Aldomedon.
1	–34.5°	0.55(49c.c.)	++	–	127	orange	23.15 (acet.)	139
2–3	34.5–45	2.13(231c.c.)	+	–	–	–	–	–
4	45–58	3.5	±	–	102	:	–	135
5	58–66	6.5	+	–	102	:	19.39	131
6	66–76.5	14.4	+	–	104	:	19.12	136
7	76.5–78	17.6	–	–	106	:	19.05	145
8–13	78–160	41.2	–	+	–	–	–	–
14	160–168	1.0	–	++	130	red	17.94	–
15	168–179	1.1	–	+	129	crange	16.83	–
16	residue	1.7	–	+	–	–	–	–

1–3 contain the ether. 7–9 contain the ethyl alcohol free from the aldehyde which was used in order to separate the ethereal layer.

The properties of several fractions are the same with those of the corresponding fractions of the saké.

The mixture of derivatives of the fraction and those of iso valeraldehyde shows no depression of melting point.

Comparison of the fraction^{14} (9 in the case of the saké) with the furfurol.

	p-nitro-ph. Color	p-nitro-ph. M. P.	Phloro glucine HCl.	Orcine HCl	Resorcine HCl (heating)
a. fraction^{14}	red	130°	reddish brown ppt	yellowish green	red
b. Furfurol	red	150°	dark green ppt.	faint blue	red

	0.5 % Vanilline in H_2SO_4	0.5 % Benzaldehyde in H_2SO_4	α-naphthol in H_2SO_2	Pyruvic acid + β-naphtylamine
a.	reddish brown	red	bluish green	red
b.	dark yellowish brown	Gray brownish violet	bluish green	red

The analytical result of the fraction^{15} resembles well with that of the methyl furfurol.

ON THE ORIGIN OF ALDEHYDES IN FERMENTATION PRODUCTS. IV.

THE ACETALDEHYDE IN THE SAKÉ.

By

MASAKAZU YAMADA.

(Received Nov. 10th., 1927)

It was shown in the previous paper that a part of the acetaldehyde in the saké originates certainly as a by-product in alcoholic fermentation from the secondary oxidation of the ethyl alcohol by microbes.(1) As to the cause of its gradual increase in storage period,(2) the action of yeasts and of some oxidizing enzymes must first be suspected as internal factors. But contrary to expectation, this is quite weak, since the yeasts are almost completely removed or destroyed by precipitation and pasteurization, and even if some, for example, aging yeasts like Willia sp. may exist, their oxidizing power of the ethyl alcohol are extremely restricted in the alcohol solution of high percentage like the saké.

The author found that the cryptomeria timber, the material of the store-pail, had a strong alcohol oxidizing power (Prof. U. Suzuki also discovered the fact.) and actually confirmed that the most part of the acet aldehyde in storage period came from the ethyl alcohol from this source. Now, the origins of the acetaldehyde in the saké will be summerised as follows :—

1. Fermentation period ; Sugar → (acetald.) → ethyl alcohol ——————→ Acetald.
   Microbes, Chiefly saké yeast (Main cause)
   Cryptomeria (one part)

2. Pasteurization ; Extract ——————→ Acetald. etc.
   heating (quite weak)

3. Storage period ; ethyl alcohol ——————→ Acetald.
   Cryptomeria (Main cause)
   Microbes and enzymes (Weak)

4. In the saké ; ethyl alcohol ——————→ Acetald.
   the same as above

It is not yet clear whether the interesting power of Cryptomeria-timber is due to the action of its chemical components or to its physical properties.

Experimental

I. Aldehyde production by saké yeast and aging yeast (Willia sp.) in

(1) M. Yamada : Bull. Agri. Ch Soc. Jap., **3**, 76–80, 1927.
(2) 〃 : J. Agri. Ch. Soc. Jap., **1**, 109–10, 1924.

the saké. 150c.c. of the saké were inoculated with the yeast-mud in the bulk of 3 grains of the soy bean.

	date		Alcohol %	Aldehyde %
1.	Control	6, July.	14.65	0.00568
2.	〃	6, Aug.	14.7	0.00572
3.	+ Saké yeast	〃	14.7	0.00859
4.	+ Willia I	〃	15.3	0.00528

In the experiments by the maceration juice or zymin (of saké yeast), analysable increase was not observed.

II. Internal cause of aldehyde production in storage period.

A little increase in the bottle though the aldehyde held by Ca-sulphite, while a marked increase in the ordinary store-pail was observed.

Variation of the aldehyde content.

	date	No. 1	No. 2	No. 3	No. 4
Original fresh saké	9, Apr.	0.00481%	0.00294%	0.00289%	0.00434%
50c.c. + 1g. of $CaSO_3$	4, Jun.	0.00558	0.00457	0.00457	0.00507
in the store-pail	7, Jun.	0.00628	0.00651	0.01077	0.01212

III. Comparison of storing in the bottle and the cask.

The volume of the bottle is about 700c.c. and that of the cask 2340c.c. The store-pail: 4500L. Variations of aldehyde content are following:

	Saké No. 4			No. 9		
date	pail	bottle	cask	pail	bottle	cask
11, Apr.	0.00449	:	:	0.00199	:	:
30, May	0.00550	0.00311	0.02139	0.00761	0.00423	0.01906
16, July	0.00563	0.00370	0.02750	0.00769	0.00461	0.02063
15, Aug.	0.00473	0.00280	0.02119	0.00836	0.00356	0.01763

p-Nitro phenylhydrazone prepared from 630c.c. of No. 4 at 16, July, melts at 127° and the M. P. of the mixture with the pure acetald.-p-nitro-ph. shows no depression. Namely the other aldehydes may not exist.

IV. Alcohol solution and cryptomeria-timber.

i. The aldehyde production in the saké or the alcohol solution with 3.5g. of chips of cryptomeria japonica for each 100c.c. of the solution, which are enclosed in a bottle.

		Alcohol solution					
	Saké	92.6%	50%	10%	5%	2%	1%
Original	0.00461	0.00339	0	0	0	0	0
After 2 days	—	0.00665	—	—	—	—	—
After 15–17 days	—	0.02022	0.02724	0.02860	0.03621	0.03308	0.01751
After a month	0.01220	0.03138	—	—	—	—	—

p-Nitro phenylhydrazone prepared from 1L. of the aldehyde free pure

17% of alcohol solution with the chips melts at 126°.

Analysis :—

Substance	N	N % (found)	N % (Calc. for $C_8H_9N_3O_2$).
0.1038 g.	21.8 c.c. (20° 759.4 m.m.)	24.09	23.46

It is clear that the acetaldehyde does not come from the chips but from the ethyl alcohol, because the distillates of the chips themselves or their water-extract do not show Rimini's reaction for the acetaldehyde.

ii. Quantity of chips in 100c.c. of 17% ethyl alcohol solution.

Chips	0.5 g.	1 g.	2 g.	5 g.
Ald. production after 17 days	0.00389 g.	0.00759	0.01479	0.03503

iii. The other conditions. The oxidation is strongly retarded in the alkaline reaction. Chips boiled in water for 30 minutes lose the power.

iv. In other alcohol solutions.

Aldehyde production in 6% of the alcohol solution with 3.5g. of the chips for each 100c.c. after 15 days.

	Methyl	n-propyl	isobutyl	n-butyl	isoamyl
Aldehyde formed	0	0.05702	0.05822	0.05037	0.02597

V. Kind of timber etc.

Solution : 100c.c. of 17% ethyl alcohol (17% is the ordinary content in the saké.)

Chips : each 3.5g.

Aldehyde formation after 15 days.

Pine 0.00949%, American pine 0.00210%, Terpen oil (1c.c.) 0.00171%, Camphor tree 0.01961%, Quercus glandulifera 0.00210%, Cherry 0.00443%, Chamaecyparis pisifera 0.01222%, White fire 0.00171%, American cryptomeria 0.00132%, Chamaecyparis obtusa, Quercus actata, Zelkova acuminata, cryptomeria oil and resin (0.5g), Paulownia imperialis, pine-resin (0.5g.), animal charcoal (1g.) and wheat bran (3.5g.) had no effect.

STUDIES ON THE CATALASE I. BEHAVIOR OF CATALASE IN THERMOPHILIC CELLULOSE FERMENTER.

By

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(Received Oct. 20th., 1927.)

This is a part of investigation which was undertaken in conjunction with

comparative study between Closteridium thermocellum (Viljoen and others)(1) and a thermophilic cellulose fermenting bacteria(2) which was isolated by the senior author (Itano). Both organisms gave a vigorous catalase action in the cellulose medium while practically no action was observed in the glucose broth. This phenomenon instigated the authors to investigate as to several factors which may influence the activity of catalase, such as composition of medium, hydrogen ion concentration, temperature, age of culture and others.

The results obtained thus far seem to indicate that:

1. The organism under investigation gives vigorous catalase action in the cellulose medium at 65°C.

2. The catalase action is inhibited in the glucose broth at 65°C while at 23°C no action takes place.

3. In the glucose broth, the concentration of hydrogen ions changes from pH 6.75 to pH 4.98 within 48 hours at 65°C, while at 23°C this change is very slight.

4. The catalase action increases with age of culture so old as sixty hours.

5. The increased hydrogen ion concentration in the glucose broth at 65°C seems to be the inhibitory factor although some other physicochemical factors may be responsible at the sametime.

As to the nature of catalase and also the inhibitory factors, the further experiments are in progress at present.

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1) J. A. Viljoen, E. B. Fred and W. H. Peterson, J, Agr. Science **16**, 1, 1926.

2) An organism which was isolated in connection with the investigaticn cn the compost.

STUDIES ON THE CATALASE. II. ROLE OF BUFFER SOLUTION IN THE CATALASE TEST.

By

Arao Itano and Satiyo Arakawa.

(Received Nov. 11th, 1927.)

In course of investigation as to the influence of hydrogen ion concentration on the catalase action, using Clark's buffer solution, it was found that the nature of buffer solution interferes with the results of titration with $KMnO_4$ as table III indicates:

TABLE III.

Titration in Buffer Solution.

No of Solution.	pH.	c.c. $KMnO_4$ used.	Difference.
4.	4.67	19.7	+ 1.3
8.	6.65	18.6	+ 0.2
14.	9.65	18.5	+ 0.1
15.	10.11	18.5	+ 0.1
H_2O only.	6.80	18.4	—

Table III. indicates that in a solution of pH 4.50 a considerably more $KMnO_4$ is taken in the titration than that of the water alone. From this result, it was thought that some salts in the solution might be interfering with the result. Consequently a set of buffer solution which was made up of M/3 H_3PO_4, M/15 Na_2HPO_4, M/3 KH_2PO_4 and found that such phosphate mixture gives uniform results as was indicated by Yamamoto.(1)

Next a buffer solution of pH 6 was prepared by taking M/5 KH–phthalate and M/5 KH_2PO_4 and M/5 NaOH, as noted in Table VI.

TABLE VI.

Titration in KH–phthalate and KH_2PO_4 Solution.

Desired pH 6.0	pH found.	c.c. $KMnO_4$ used.	Difference.
M/5 KH-phthalate and M/5 NaOH	5.90	18.10	+ 1.45
M/5 KH_2PO_4 and M/5 NaOH	5.90	16.75	+ 0.10
H_2O	6.80	16 65	—

Table VI indicates clearly that the presence of KHphthalate effect the titration by taking up more $KMnO_4$ than in the case of water alone.

From these results, it was concluded that the catalase test by titration with permanganate is influenced markedly by the presence of KHphthalate in the mixture while the phosphate mixture gives very uniform results. Further investigation as to the influence of hydrogen ion concentration on the activity of catalase will be reported as soon as the results are obtained.

(1) Yamamoto, Y. (Ziozogaku Zasshi (J. of Zymology) 5, 2, 79, 1927.

STUDIES ON THE CATALASE
III. INFLUENCE OF HYDROGEN ION CONCENTRATION AND OTHER FACTORS ON THE CATALASE.

By

ARAO ITANO and SATIYO ARAKAWA.

(Received Nov. 11th., 1927.)

The critical and optimum concentration of hydrogen ion for the catalase were determined by using the phosphate mixture which was found to serve the purpose satisfactorily. Besides the hydrogen ion concentration, the influence of several other factors, such as quality and quantity of glucose, the age of culture, period of reaction, quantity of catalase and velocity constant, temperature and some chemical salts upon the catalase activity, was investigated.

The authors reached the following summary and conclusions:

1. The buffer solutions, phosphate mixture used in this investigation, give uniform results in the permanganate titration for the catalase test.

2. The titration with permanganate gives finer data than the nitrometer method.

3. pH 8.64 is the optimum reaction for the catalase in question.

4. The acid reaction pH$<$7 has stronger depressive action than the alkaline or pH$>$7.

5. The quality and quantity of glucose added so far as this investigation is concerned, there is no appreciable difference on the catalase. In all cases, the catalase action was depressed.

6. Seventy two hours old culture gave most vigorous catalase action and as the culture grew older, the action was declined. This may be due to an accumulation of metabolic products and also disappearance of the catalase itself.

7. The velocity of catalase action decreases on standing which may be due to the poisonous effect of H_2O_2 or interference of molecular O_2 produced in the titration system.

8. The velocity constant is proportional to the concentration of the catalase. It is a typical unimolecular reaction.

9. The catalase in question resists higher temperature than generally known temperature for other bacterial catalase viz. thirty minutes heating at 60°C is the optimum; even 80 or 100°C do not destroy the action entirely.

10. $MgSO_4$ and $CuSO_4$ have very slight depressive influence on the catalase action while KCN, HCl and $HgCl_2$ have very marked effect among which KCN was the strongest.

(Further investigation as to the nature of enzyme and absorption phenomenon is in progress.)

ON THE SEPARATION OF GLYCERIDES.

PART I. LINSEED OIL.

By

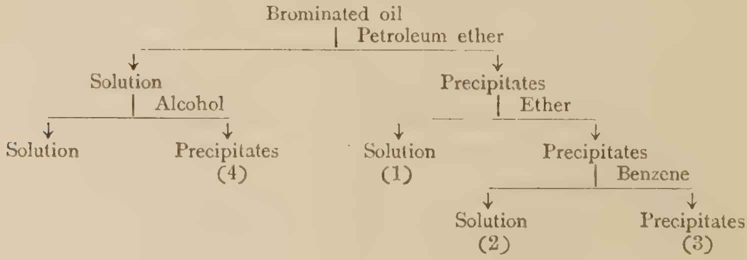
Bunsuke Suzuki and Yoshikuni Yokoyama.

(Received Nov. 6th., 1927.)

Former studies on constituents of fats and oils have usually dealt with the isolation and identification of fatty acids, if possible quantitatively. This is sufficient to account for the nature of fats and oils if they be composed of simple glycerides, but there are many indication that the principal components of fats and oils are mixed glycerides and in reality not a few of them have been isolated by different chemists. Unless we assume the presence of mixed glycerides we cannot account for the difference of nature which exists between cocoa butter and mutton tallow, both being composed of nearly the same quantity of the same kinds of fatty acid. If fats and oils are composed of mixed glycerides, it is of primary importance to isolate glycerides as such in order to know what fats and oils are.

Taking advantage of the different solubilities of brominated glycerides against different solvents, one of the authors (S.), in co-operation with several co-workers, has up to the present, succeeded in isolating more than 20 glycerides from 6 fats and oils though some of the experiments are too incomplete to publish as yet. There is nothing new about the process, for the same method has been applied previously for the same purpose by several different chemists. But these investigators adopted the process of fractional precitation while we used different solvents to separate glycerides and we found our process the better.

The outline of our process undertaking for the separation of glycerides of linseed oil may be given in the following scheme.



The brominated glycerides when separated are not crystalline when inspected under a microscope. To insure purity, therefore, each fraction was

reprecipitated from a suitable solvent several times until it gave a constant composition and melting point. After the bromine content and melting point were determined the glycerides were hydrolized with hydrochloric acid, and the resultant acids were identified and weighed to ascertain their molecular proportion in the glyceride.

The following glycerides were isolated from linseed oil.

		m. p.
1.	Dilinoleo-linolenin bromide	78°C.
2.	Linoleo-dilinolenin bromide (a)	117–118°C.
3.	Linoleo-dilinolenin bromide	158°C.
4.	Dilinoleo-olein bromide	oil

The figures correspond to those of the above mentioned scheme.

2 and 3 are isomeric to each other.

Experimental

Bromination 200g. of linseed oil was dissolved in Il. of petroleum ether and dried by adding a sufficient quantity of anhydrous sodium sulfate. To the dry ethereal solution of the oil, bromine was added from a burette, under constant stirring and thorough cooling until the color of bromine did not disappear. The stirring continued for several hours and finally the precipitates were gathered on a Buchner's funnel followed by thorough washing with a mixture of dry petroleum ether and glacial acetic acid.

1. Dilinoleo-linolenin bromide The ethereal solution of the brominated oils, which were with difficulty soluble in petroleum ether, evaporated to dryness. The white powder thus obtained was treated repeatedly with ether until the melting point and bromine content remained constant. The bromine was determined by Baubigny and Chavanne's method. The bromide melted at 78°C, Br: 56.21%. The bromine content corresponds to 14 atoms of the element in one mole of the glyceride. It is probable, therefore, that bromine atoms are contented in pairs of $2+2\times6$ or $2\times4+6$.

To determine which one of the two pairs really occurs in the glyceride, one part of the bromide was hydrolized with ten parts of hydrochloric acid. The resultant brominated fatty acids were separated into two parts, one easily soluble in ether and the other with difficulty soluble. Easily soluble acid, m. p.: 115°C, Br: 53.27% (53.33% as tetrabromostearic acid). Soluble with difficulty acid, m. p.: 179°C. Br: 63.59% (63.32% as hexabromostearic acid). The molecular proportion of the acids was 2.29 : 1.

2. Linoleo-dilinolenin bromide With difficulty soluble in ether but easily soluble in benzene. M. p.: 117–118°C, Br: 59.32% $(0+2\times8$ or $4+2\times6)$ Tetrabromostearic acid, m. p.: 115°C, Br: 53.81% Hexabromostearic acid,

m. p.: 179°, Br: 63.64%, molecular proportion of the acids was 1 : 1.91.

3. Linoleo-dilinolenin bromide With difficulty, soluble in nearly all fat solvents. M. p.: 158°C, Br: 59.32%. By hydrolysis with hydrochloric acid tetrabromostearic acid (m. p.: 115°C, Br: 53.76%) and hexabromostearic acid (m. p.: 179°C, Br: 63.45%) were obtained in molecular proportion of 1 : 2.03.

It is worthy of notice that two isomerides of linoleo-dilinolenin bromide (2 and 3) differ markedly as to the melting point and behavior against solvents. If one of the isomerides has a symmetrical, the other must have a nonsymmetrical construction, being thus optically active. The rotatory powers of the two were estimated but both happened to be inactive or the activity was so slight that it was difficult to decides which one was really active.

4. Dilinoleo-olein bromide Soluble in petroleum ether but with difficulty soluble in hot alcohol. An oil, Br: 47.25% ($6+2\times2$ or $2+2\times4$). By acid hydrolysis, dibromostearic acid (Br: 36.43%) and tetrabromosteacic acid (m. p.: 115°C, Br: 53.79%) were obtained in molecular proportion of 1 : 1.86.

PART II. SOY BEAN OIL.

Soy bean oil was treated in ways similar to those in the case of the linseed oil. The previous scheme.

The following brominated glycerides were isolated and identified.

		m. p.
1.	Dilinoleo-linolenin bromide	78°C
2.	Linoleo-dilinolenin bromide	118°C
3.	Trilinolenin bromide	166°C
4.	Dilinoleo-olein bromide	oil

Experimental

1. Dilinoleo-linolenin bromide With difficulty soluble in petroleum ether but easily soluble in ether. M. p.: 78°C, $(\alpha)_D^{20}$: 0, Br: 56.36% (Calc.: 56.06). By hydrolysis with hydrochloric acid, tetrabromostearic acid (m. p.: 178°C, Br: 64.04%) were obtained in molecular proportion of 1.80 : 1.

2. Linoleo-dilinolenin bromide Soluble in benzene but with difficulty soluble in ether. M. p.: 118°C, Br: 59.89% (Calc.: 59.37). By hydrolysis with hydrochloric acid, tetrabromostearic acid (m. p.: 113°C, Br: 53.95%) and hexabromostearic acid (m. p.: 178°C, Br: 62.93%) were obtained in molar proportion of 1 : 2.2.

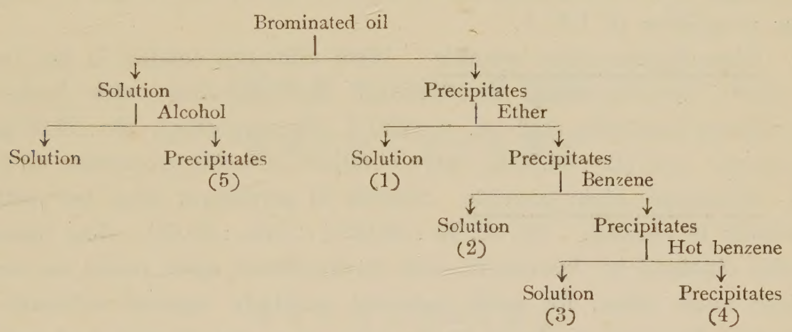
3. Trilinolenin bromide With difficulty soluble in cold but soluble in hot benzene. Crystalline plates (from carbon tetrachloride). M. p.: 166°C, Br: 61.99% (Calc.: 62.23). By hydrolysis with hydrochloric acid, hexabro-

mostearic acid, (m. p. : 178°C, Br : 63.91%) and no other acid, was obtained.

4. Dilinoleo-olein bromide Soluble in petroleum ether but with difficulty soluble in alcohol. An oil at room temperature. Br : 47.80 (Calc. : 47.56). By hydrolysis with hydrochloric acid, tetrabromostearic acid (m. p. 113°C, Br : 53.48) and dibromostearic acid [Br : 36.64 (Calc. : 36.18%)] were obtained in molar proportion of 2.03 : 1.

PART III. TRAIN OIL.

Train oil was treated in ways similar to the cases of part I and II.



The following brominated glycerides were isolated and identified :

		m. p.
(1)	Arachidono-clupanodono-olein bromide	95°C
(2)	Diclupanodono-olein bromide	132°C
* (3)	Diarachidono-olein bromide	200°C (decomposing)
* (4)	Diarachidono-olein bromide	216°C (decomposing)
(5)	Dizoomaro-olein bromide	oil

* Isomeric to each other.

Experimental

The brominated oil was too viscous to allow the use of any filter so that precipitates were separated by centrifuging.

(1) Arachidono-clupanodono-olein bromide Soluble in ether but with difficulty soluble in petroleum ether. M. p. : 95°C, Br : 61.98% (Calc. : 62.60). The brominated fatty acids, obtained by hydrolysing the glyceride with hydrochloric acid, were treated with alcohol and the larger portions of them went into solution while a part remained in residue. The alcoholic solution was evaporated to dryness and the residue was treated with petroleum ether dividing it into two parts, one soluble and the other insoluble. Octabromo-arachidic acid, m. p. : 220°C (decomposing), Br : 66.50% (Calc. : 66.70). Deca-bromobehenic acid, m. p. : 161–162°, Br : 69.98% (Calc. : 70.75). Dibromostearic acid, an oil, Br : 36.52% (Calc. : 36.18). Molar proportion of three acids, 1 :

1.05 : 1.28.

(2) Diclupanodono-olein bromide Soluble in cold benzene but with difficulty soluble in ether. M. p.: 132°C, Br : 64.03 (Calc. : 64.19). Br hydrolysis with hydrochloric acid, decabromobehenic acid (m. p.: 161–162°C, Br : 69.67%) and dibromostearic acid (Br : 36.79%) were obtained in molar proportion of 1.83 : 1.

(3) Diarachidono-olein bromide With difficulty soluble in cold but soluble in hot benzene. M. p.: 200°C (decomposing), Br : 60 : 60.69% (Calc.: 60.76). By hydrolysis with hydrochloric acid, octabromoarachidic acid [m. p.: 220 (decomposing), Br : 67.01%] and dibromostearic acid (Br : 36.76%) were obtained in molar proportion of 1.8 : 1.

(4) Diarachidono-olein bromide With difficulty soluble in hot benzene. M. p.: 216°C (decomposing), Br : 61.23%. By hydrolysis with hydrochloric acid, octabromoarachidic acid [m. p.: 220°C (decomposing), Br : 67.07%] and dibromostearic acid (Br : 36.53%) were obtained in molar proportion of 1.85 : 1.

(5) Dizoomaro-olein bromide Soluble in petroleum ether but with difficulty soluble in alcohol. An oil. Br : 36.92% (Calc. : 36.64). The brominated fatty acids obtained by hydrolysis with hydrochloric acids could not be separated from each other, for both behaved similarly against solvents. The mixture was dissolved in glacial acetic acid, an excess of zinc dust was added to the solution and, provided with a reflux condenser, the flask was heated on a water bath. The contents of the flask were filtered, the filtrate was evaporated to dryness and the residue was dissolved in ether. A mixture of unsaturated fatty acids was obtained by evaporating the ethereal solution to dryness. The mixture was dissolved in a dilute soda solution and oxidized with a potassium permanganate solution (1.5%) under constant stirring and through cooling. The precipitates of mangan oxide were dissolved by passing sulphur dioxide and oxy acids, which floated on the surface of the solution, were gathered on a Buchner's funnel, recrystallized from alcohol and treated with ether. Part (dioxypalmitic acid) of them went into ethereal solution while the other (dioxystearic acid) remained as residue. Dioxypalmitic acid melted at 115°C. Dioxystearic acid melted at 132–133°C.

(College of Agriculture, Kyoto Imperial University.)

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